heated to 1600°, quenched in mercury, and sawed open. The walls had been attacked somewhat, and in the interior of the solidified thorium tetrafluoride a few gray areas of finely divided thorium metal were found.

No evidence for the existence of a lower fluoride of thorium was found. There was some indication however, that thorium dissolves slightly in its fluoride at high temperature, a behavior similar to that of uranium in its tribromide,[6] the alkaline earth metals in their halides,[7] or cerium in its chloride.[8]

Mention should be made of the preparation of thorium(III) and thorium(II) halides[9-11] prepared by reduction of the tetrahalides, especially the iodide, by the metal. These lower iodides were observed to parallel the corresponding halides of zirconium and hafnium in physical and chemical properties. Similarly a brown thorium(III) and silvery thorium(II) sulfide are known[12] whose properties indicate the presence of no f electrons.

(6) C. D. Thurmond, Plutonium Project Report CC-2522 (Dec. 20, 1944).

(7) D. D. Cubicciotti and C. D. Thurmond, THIS JOURNAL, **71**, 2149 (1949).

(8) D. D. Cubicciotti, *ibid.*, **71**, 4119 (1949).

(9) E. Hayek and Th. Rehner, *Experientia*, **5**, 114 (1949).

(10) E. Hayek, Th. Rehner, and A. Frank, *Monatsh.*, **82**, 575 (1951).

(11) J. S. Anderson and R. W. D'Eye, *J. Chem. Soc.*, (Suppl. Issue No. 2), S 244 (1949).

(12) E. D. Eastman, *et al.*, THIS JOURNAL, **72**, 4019 (1950).

CONTRIBUTION NO. 52 FROM THE
INSTITUTE FOR ATOMIC RESEARCH
IOWA STATE COLLEGE
AMES, IOWA

RECEIVED OCTOBER 30, 1951

COMMUNICATIONS TO THE EDITOR

THE REACTION OF DI-ISOPROPYL FLUOROPHOSPHATE WITH TYROSINE

Sir:

We have been engaged for some time on a comparative study of the action of di-isopropyl fluorophosphate (DFP) and di-isopropyl chlorophosphate (DClP) on amino-acids under "physiological" conditions in the hope of finding some clear-cut difference which might parallel the marked difference between the biochemical activities of these two compounds[1] (DFP is toxic and inhibits cholinesterase and other enzymes whereas DClP is not markedly toxic). The appearance of the recent paper of Wagner-Jauregg, O'Neill and Summerson[2] on a similar subject prompts us to make this preliminary communication of our positive findings.

Wagner-Jauregg, O'Neill and Summerson[2] studied the action of non-polar DFP and DClP on a number of amino-acid esters and amines in that DClP is the more reactive solution and uniformly observed the latter to be the more reactive[3]; they were unable to phosphorylate amino-acids in slightly alkaline aqueous media with DFP although they observed, but do not describe in detail, reaction between DFP and phenol in aqueous potassium carbonate.

We have been more fortunate and have been able to demonstrate a marked difference in the reactivity of DFP and DClP toward tyrosine. Using 0.02 *M* halophosphate and 0.005 *M* tyrosine in 0.08 *M* sodium bicarbonate at 38° and following the disappearance of free phenolic hydroxyl by the colorimetric method of Thomas[4,5] we obtained the following results

Time hr.	3	4	9	24
% Reaction of (DFP)	43	49	52	56
phenolic OH. (DClP)	-	7	10	8

Clearly DFP reacts readily, under these conditions, with the phenolic hydroxyl group of tyrosine whereas DClP does not. The reaction product from DFP, O-di-isopropylphosphoryl-tyrosine (I), was isolated from the reaction mixture, by chromatography on deactivated charcoal,[6] as needles, m.p. 158–160° (dec.), from aqueous acetone (Found N, 4.1; $C_{15}H_{24}O_6NP$ requires N, 4.1), and, more easily, as its N-2,4-dinitrophenyl derivative, needles, m.p. 159–160°, from methanol (Found C, 49.4; H, 5.1; N, 7.9; $C_{21}H_{26}O_{11}N_3P$ requires C, 49.3; H, 5.1; N, 8.2). The structure of (I) was confirmed by its chromatographic recognition in the hydrogenation product of its amorphous *p*-bromocarbobenzoxy derivative (Found C, 49.7; H, 5.0; N, 2.8; $C_{23}H_{29}O_2NBrP$ requires C, 49.5; H, 5.2; N, 2.5), obtained by treating DFP similarly with N-*p*-bromocarbobenzoxytyrosine, m.p. 156–157° (Found: C, 51.7; H, 4.1; N, 3.4; $C_{11}H_{16}O_5NBr$ requires C, 51.8; H, 4.1; N, 3.55).

This marked difference in the chemical behavior of the two halophosphates suggests, although it does not prove, that the reaction of DFP with cholinesterase and other sensitive enzymes involves reaction at a tyrosine side-chain; it is of interest that chymotrypsin, which is sensitive to DFP, has been

(1) E. C. Webb, *Biochem. Soc. Symp.*, **2**, 50 (1948); H. G. Cook, B. C. Saunders and F. E. Smith, *J. Chem. Soc.*, 635 (1949).

(2) T. Wagner-Jauregg, J. J. O'Neill and W. H. Summerson, THIS JOURNAL, **73**, 5202 (1951).

(3) Cf. B. C. Saunders and G. J. Stacey, *J. Chem. Soc.*, 695 (1948).

(4) L. E. Thomas, *Arch. Biochem.*, **5**, 175 (1944).

(5) The absorptiometer used was purchased with the aid of a grant from the Central Research Fund of the University of London for which we express our thanks.

(6) Schramm and J. Primosigh, *Ber.*, **76**, 373 (1943).

stated[7] to require a tyrosine side-chain for activity. We are at present engaged in studying the action on tyrosine of other choline-esterase inhibitors and in attempts to isolate O-phosphorylated tyrosine from the reaction products of suitable choline-esterase inhibitors with chymotrypsin and other sensitive enzymes. Full details of our work will be published elsewhere in due course.

(7) I. W. Sizer, *J. Biol. Chem.*, **160**, 547 (1945).

IMPERIAL COLLEGE OF
SCIENCE AND TECHNOLOGY, R. F. ASHBOLT
LONDON S. W. 7, ENGLAND H. N. RYDON

RECEIVED JANUARY 14, 1952

THE EXCHANGE REACTION BETWEEN COBALTOUS AND COBALTIC IONS IN PERCHLORIC ACID SOLUTION

Sir:

Previous investigators[1] found that under their conditions the rate of electron transfer between cobaltous and cobaltic ions was complete within the time of separation. Since the possibility existed that their results were due to exchange induced by the separation method, we have reinvestigated the exchange using a non-precipitation method.

We find that, at low cobalt concentrations, the rate is measurable. In our experiments, separation of the cobaltous and cobaltic species was effected by adding the exchange mixture to an ammoniacal solution of sodium Versenate,[2] acidifying the resulting solution with HNO_3, adding NH_4CNS and extracting the cobalt (II) with methylisobutyl ketone. Each fraction was then converted to a cobalt (III) "Versenate" complex for gamma counting and spectrophotometric analysis. Early experiments established that the separation method gave satisfactory activity and material balances (100 ± 5%) when both fractions were examined. In later work, only the specific activity of the Co (III) fraction was measured since this, together with the infinite time specific activity is sufficient to determine the fraction exchange. About 30 or 40% of the Co(III) was reduced to Co(II) during the separation, but a negligible amount of reduction occurred in the exchange mixture before separation. The amount of induced exchange was large (*ca.* 20%) but fairly reproducible.

Co^{+3} was prepared by electrolysis of a perchloric acid solution of cobaltous perchlorate.

The tracer was Co^{60} obtained from Oak Ridge. Experiments were usually done with cobaltous tracer, but one experiment using cobaltic tracer gave results which were consistent with the other data.

The exchanges reported in Table I were carried out in the dark, although experiments showed that no appreciable effects were caused by light of ordinary laboratory intensity.

An experiment done in a vessel packed with glass beads indicated that catalysis by glass surfaces is negligible.

(1) S. A. Hoshowsky, O. G. Holmes and K. J. McCallum, *Can. J. Research*, **27B**, No. 4, 258 (1949).

(2) The sodium salt of ethylenediaminetetracetic acid, manufactured by Bersworth Chemical Co.

The data for several of our experiments are given in Table I. All runs were made at 0° and in 1 *M* $HClO_4$. The reaction obeyed the usual exponential rate law with four or five points on each curve. The constancy of the product of half-time and total cobalt concentration shows the reaction to be second order, presumably first order in each of the two cobalt ions. The average bimolecular rate constant is calculated to be 46 liter-mole^{-1} min.$^{-1}$.

TABLE I

ELECTRON TRANSFER BETWEEN COBALTOUS AND COBALTIC IONS AT 0° AND 1 *M* $HClO_4$

Co(molarity × 10^3) Total	Co^{+2}	Co^{+3}	$T_{1/2}$ (min.) (±0.5 min.)	$T_{1/2}$ × total Co(× 10^3)
0.717	.124	.593	22.0	1.58
1.33	.14	1.19	11.5	1.53
1.47	...	...	10.8	1.59[a]
2.93	.27	2.66	4.8	1.41
3.03	~1.5	~1.5	4.8	1.45[b]
				Av. 1.51

[a] Glass beads added. [b] Tracer added as Co^{+3}.

Experiments are under way to investigate the induced exchange and to study the kinetics of the reaction in detail.

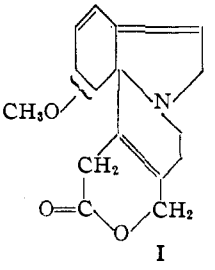
DEPARMENT OF CHEMISTRY
CORNELL UNIVERSITY NORMAN A. BONNER
ITHACA, NEW YORK JOHN P. HUNT

RECEIVED FEBRUARY 25, 1952

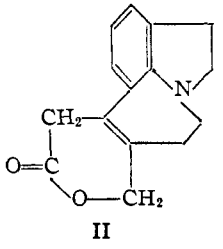
THE STRUCTURES OF β-ERYTHROIDINE AND APO-β-ERYTHROIDINE[1]

Sir:

Previously we have suggested partial structures for apo-β-erythroidine and certain other derivatives.[2,3] Additional evidence, which we are now presenting, makes it possible to assign structures I and II to β-erythroidine and apo-β-erythroidine, respectively.



I



II

Apo-β-erythroidine, a dihydroindole derivative, contains a δ-lactone ring, has no terminal methyl group and yields 7-carboxyisatin on oxidation.[2] These results indicate a tricyclic nucleus having fused five-, six- and seven-membered rings. Hofmann degradation studies have now demonstrated the presence of *two* —CH_2—CH_2— groups attached to the nitrogen atom, making it necessary to place the lactone ring as shown. The evidence for this is the appearance in the Hofmann degradation products of the characteristic absorption peaks in the infrared associated with the —CH=CH_2

(1) Aided by a grant from the United Cerebral Palsy Association.

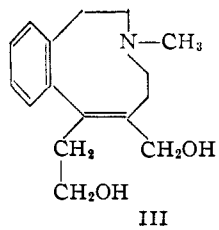
(2) M. F. Grundon and V. Boekelheide, THIS JOURNAL, in press.

(3) V. Boekelheide and E. Agnello, *ibid.*, **73**, 2286 (1951).

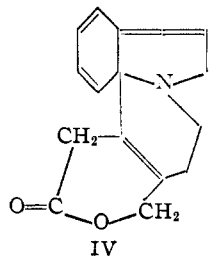
group.[4,5] Thus, des-N-methyl-apo-β-erythroidine, $C_{16}H_{17}NO_2$ (m.p. 167.5–169°, found C, 75.06; H, 6.71) gives formaldehyde on ozonolysis and shows a peak at 10.82 μ which is absent in its dihydro derivative, $C_{16}H_{19}NO_2$ (m.p. 130–130.5°, found C, 74.95; H, 7.71; —C—CH_3, 5.58). Likewise, the Hofmann degradation product of the dihydro derivative (found C, 74.92; H, 7.98) has a peak at 11.00 μ which is absent in its hydrogenation product, $C_{17}H_{23}NO_2$ (found C, 74.22; H, 8.46).

Structure I for β-erythroidine can be deduced from new evidence establishing III as the structure of the Hofmann degradation product of dihydro-β-erythroidinol.[3] This product undergoes slow hydrogenolysis of the allylic hydroxyl to yield a desoxy derivative, $C_{16}H_{23}NO$ (m.p. 89–90.5°, found C, 78.24; H, 9.36; —C—CH_3, 2.66). Further Hofmann degradation of the desoxy derivative gives an oil, $C_{17}H_{25}NO$ (found C, 78.48; H, 9.64), having peaks at 10.03 and 11.02 μ which are absent in the corresponding dihydro derivative, $C_{17}H_{27}NO$ (found C, 77.99; H, 10.50). This dihydro derivative, on a further Hofmann degradation, yields a nitrogen-free product, $C_{15}H_{20}O$ (found C, 82.78; H, 9.24) having peaks at 10.01 and 11.10 μ which are again absent in its hydrogenated derivative, $C_{15}H_{22}O$ (found C, 82.30; H, 10.19). Ozonolysis of this final hydrogenation product yields methyl ethyl ketone, whose identity is established by comparison of its 2,4-dinitrophenylhydrazone with an authentic sample. The presence of two —CH_2—CH_2— groups attached to the nitrogen supports the conclusion made previously with apo-β-erythroidine, and the isolation of methyl ethyl ketone shows the arrangement of the lactone ring with respect to the nitrogen.

Finally, when des-N-methyl-dihydro-β-erythroidinol (III) is subjected to exhaustive methylation without prior hydrogenation, a nitrogen-free product, $C_{15}H_{18}O_2$ (m.p. 84–86°, found C, 78.18; H, 7.58) results which readily gives a tetrahydro derivative, $C_{15}H_{22}O_2$ (found C, 76.79; H, 9.56). Permanganate oxidation of this tetrahydro derivative gives *o*-ethylbenzoic acid, identified by comparison of its infrared spectrum with that of an authentic sample, and establishes III as the correct structure.



III



IV

Since β-erythroidine serves as precursor for both II and III, structure I would appear to be the only logical possibility for it. This formulation shows for the first time the close chemical relationship between β-erythroidine and the other erythrina alkaloids.[6] Recent suggestions[7,8a,b] regarding the structure of β-erythroidine find no support in our experiments.[9]

(4) D. Barnard, L. Bateman, A. J. Harding, H. P. Koch, N. Sheppard and G. B. Sutherland, *J. Chem. Soc.*, 915 (1950).

(5) Because apo-β-erythroidine and certain of its derivatives show absorption in the 10 μ region, only the terminal methylene peak in the 11 μ region is informative for the Hofmann degradation products of this series.

(6) G. W. Kenner, H. G. Khorana and V. Prelog, *Helv. Chim. Acta*, **34**, 1969 (1951); M. Carmack, B. C. McKusick and V. Prelog, *ibid.*, **34**, 1601 (1951).

(7) C. Lapiére and R. Robinson, *Chem. and Ind.*, **30**, 650 (1951).

(8) (a) F. Koniuszy and K. Folkers, THIS JOURNAL, **73**, 333 (1951); (b) **72**, 5579 (1950).

(9) Although the methylation and oxidation experiments on desmethoxy-β-erythroidine reported by Koniuszy and Folkers (ref. 8b) are not in accord with our formulation, we have repeated their experiments and, in our hands, phthalic acid was isolated in 36% yield as the only oxidation product. Since desmethoxy-β-erythroidine, to which we assign structure IV, would be expected to undergo Hofmann degradation under the conditions employed for methylation, phthalic acid is a rational product of oxidation.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF ROCHESTER
ROCHESTER, NEW YORK

V. BOEKELHEIDE
M. F. GRUNDON
J. WEINSTOCK

RECEIVED MARCH 13, 1952

PHOSPHONOUS AND PHOSPHONIC CATION EXCHANGE RESINS

Sir:

Cation exchange resins available up to the present time have consisted of three major types—those in which sulfonic, carboxylic, or phenolic acid groups have served as the source of the exchangeable cations. There have now been developed in this laboratory phosphonous[1] and phosphonic[1] cation exchange resins which show certain desirable chemical characteristics that are not found in any of the older exchangers.

Figure 1 shows the titration curve as obtained in 1 *N* sodium chloride solution by the "Direct Titration" method of Gregor and Bregman,[2] for a phosphonic exchanger having a total capacity of 8.8 meq./g. dry resin. It can be seen that the first ionization occurs at a *p*H slightly higher than that of a sulfonic acid resin, while the second one is at a *p*H intermediate between the carboxylic and phenolic types.[2] The *pK* valuesin dicated from this curve compare favorably with those found by Rumpf and Chavane[3] for aliphatic phosphonic acids. The phosphonous resins show titration curves identical with the first portion of the phosphonic type.

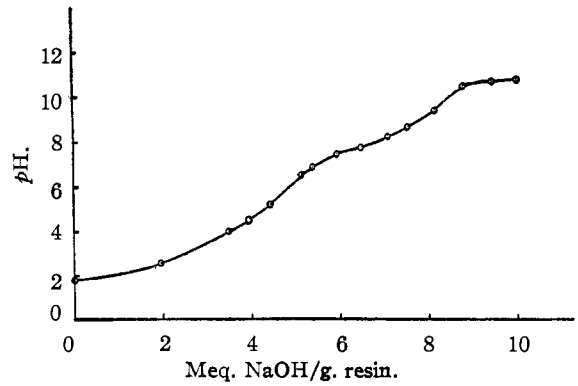


Fig. 1.—Titration curve of phosphonic acid cation exchange resin.

(1) Nomenclature follows that of Chemical Abstracts.

(2) H. P. Gregor, and J. I. Bregman, THIS JOURNAL, **70**, 2370 (1948).

(3) P. Rumpf, and V. Chavane, *Compt. rend.*, **224**, 919 (1947).

These resins are the first ones known to show a selectivity for sodium over potassium. This phenomenon and the subsequent inversion of the order of selectivity for the alkali cations as compared to sulfonic resins may be explained by a consideration of the polarizability of ionic groups and water as calculated by Teunissen and Bungenberg de Jong.[4] They found the order of polarizability to be: phosphate > water > sulfate. Since the order of polarizing ability of the alkali cations is Li > Na > K it would be expected that whereas in the sulfonic acid resins the volume sequence, as has been shown by Gregor, Gutoff and Bregman,[5] is Li > Na > K and consequently the order of preference for the alkali cations is K > Na > Li, in the case of the phosphonic resin the volume sequence should be K > Na > Li and the order of selectivity Li > Na > K. These volume and selectivity orders are in accord with values found in this laboratory for these resins and consequently the pressure-volume selectivity theory as expounded by Gregor[6] may be considered to apply to these systems when modified by the Teunissen–Bungenberg de Jong polarizability considerations. A detailed discussion of the experimental data together with the extension of this theory to carboxylic exchange resins will be given in a forthcoming paper.[7]

These resins show a volume increase of about 50% on going from the hydrogen to the sodium state. They are orange-yellow in the hydrogen state but show a striking color change to dark brown when placed in any of the alkali metal states.

Potential applications of phosphonous and phosphonic resins as a result of their unique properties include sodium depletion in physiological applications, rare earth separations, and use in mixed bed and reverse demineralization units.

(4) P. H. Teunissen and H. G. Bungenberg de Jong, *Kolloid Beihefte*, **48**, 80 (1938).
(5) H. P. Gregor, Fradelle Gutoff, and J. I. Bregman, *J. Colloid Science*, **6**, 3, 245 (1951).
(6) H. P. Gregor, THIS JOURNAL, **73**, 642 (1951).
(7) J. I. Bregman, and Y. Murata, in preparation.

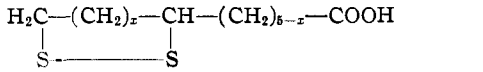
NATIONAL ALUMINATE CORPORATION J. I. BREGMAN
CHICAGO, ILLINOIS YOSHIAKI MURATA

RECEIVED JANUARY 17, 1952

STRUCTURE OF PROTOGEN-A

Sir:

An unidentified growth factor for *Tetrahymena geleii* was first described in 1944 by Kidder and Dewey.[1] Using the test described by these workers it was found that several substances would produce a response in the organism.[2] These factors were called the "protogens."[2,3] The isolation of the sulfur-containing compound protogen-B from liver was described recently.[3] This compound (I) upon titration with sodium hydroxide was found to have a molecular weight corresponding to about 230 on the basis of one carboxyl group per molecule. Elementary analysis indicated the presence of 8 carbon and 2 sulfur atoms. When saponified with an excess of sodium hydroxide, I was converted to another compound (II) which gave a positive nitroprusside reaction and which contained one –SH group as indicated by titration with iodine. Reduction of I with sodium borohydride yielded a third compound (III) which contained two –SH groups as shown by iodine titration. Mild oxidation readily converted III to a disulfide (IV) as indicated by the disappearance of the nitroprusside reaction and by its reappearance on treatment with cyanide. Protogen-A[3] also gave a nitroprusside reaction after treatment with cyanide. A band at 1040 cm.$^{-1}$, present in the infrared absorption spectrum of protogen-B and indicating a sulfoxide group, was absent from the spectra of IV and of protogen-A. Protogen-A and IV appeared to be identical as shown by biological activity, liquid–liquid countercurrent distribution, paper chromatography and infrared absorption spectra. The absence of C-methyl groups in I was indicated by a negative Kuhn–Roth determination. Upon treatment of I with Raney nickel,[4] octanoic acid was obtained and identified by means of its infrared absorption spectrum, its melting point, and the X-ray powder photograph of its S-benzylthiuronium salt. These findings showed the probability of the following structure for IV, I being presumed to be a



sulfoxide. By the use of molecular models, a stable ring could be constructed for $x = 2$. The name "thioctic acid" is proposed for this structure ($x = 2$), a sulfur-containing organic acid with 8 carbon atoms. The synthesis of DL-thioctic acid with biological activity corresponding to that of the "protogens"[2] and the "lipoic acids"[5] is described in another communication.[6] Numerical prefixes indicating the position of the carbon atom to which the secondary sulfur is attached may be used to designate compounds in this series with different values for x.

(1) G. W. Kidder and V. Dewey, *Biol. Bull.*, **87**, 121 (1944).
(2) E. L. R. Stokstad, *et al.*, *Arch. Biochem.*, **20**, 75 (1949).
(3) E. L. Patterson, *et al.*, THIS JOURNAL, **73**, 5919 (1951).
(4) R. Mozingo, *et al.*, *ibid.*, **65**, 1013 (1943).
(5) L. J. Reed, *et al.*, *Science*, **114**, 93 (1951).
(6) M. W. Bullock, *et al.*, THIS JOURNAL, **74**, 1868 (1952).

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LEDERLE LABORATORIES DIVISION E. L. R. STOKSTAD
AMERICAN CYANAMID COMPANY E. L. PATTERSON
PEARL RIVER, NEW YORK J. V. PIERCE
MARY MACCHI
FLORA P. DAY

RECEIVED DECEMBER 26, 1951

SYNTHESIS OF *DL*-THIOCTIC ACID

Sir:

Thioctic acid,[1] a compound with the biological activity of protogen, was synthesized as follows: furylacrolein was hydrogenated to 2-tetrahydrofurylpropanol (I) over Raney nickel.[2] I was converted to 2-tetrahydrofurylpropyl chloride (II) with thionyl chloride and II was converted to γ-(2-tetrahydrofuryl)-butyric acid (III).[3] III was converted to a mixture of 5-hydroxy-8-iodocaprylic

(1) J. A. Brockman, Jr., *et al.*, THIS JOURNAL, **74**, 1868 (1952).
(2) A. Hintz, *et al.*, *Ber.*, **76**, 676 (1943).
(3) H. Gilman and H. P. Hewlett, *Rec. Trav. Chim.*, **51**, 93 (1932).

acid (IV) and its lactone by cleavage of the tetrahydrofuran ring with a solution of potassium iodide in 95% phosphoric acid. The crude mixture containing IV and its lactone was heated at reflux for sixteen hours with 2 moles of thiourea and 1.5 moles of hydrobromic acid (34%) and the resultant reaction mixture was hydrolyzed with 0.5 *N* sodium hydroxide at 100° for 15 minutes. The solution was acidified and extracted with chloroform. The chloroform solution was then treated with a slight excess of aqueous potassium iodide–iodine solution, chloroform was removed by distillation and the residue was purified by chromatography on silicic acid to yield a yellow oil, neutralization equivalent = 208. The biological activity of the oil was approximately 20% that of protogen-A for *Tetrahymena geleii* and a species of *Corynebacterium*.[4] Oxidation of the oil with *t*-butyl hydroperoxide yielded a second biologically active compound with properties closely similar to those of protogen-B as measured by paper chromatography, solvent distribution and infrared studies. This compound gave a crystalline S-benzylthiuronium salt, m.p. 143 to 144°, calculated for $C_{16}H_{24}N_2S_3O_3$: C, 49.45; H, 6.23; N, 7.21; S, 24.76; found C, 49.81; H, 6.30; N, 7.31; S, 25.39; C-methyl, negative. The position of the secondary sulfur atom cannot be stated unequivocally, as migration of the hydroxyl group has been shown to occur in aliphatic hydroxy-acids when treated with heat and acid.[5]

(4) E. L. R. Stokstad, *et. al.*, *Proc. Soc. Exp. Biol. Med.*, **74**, 571 (1950).

(5) E. E. Blaine and A. Kohler, *Compt. rend.*, **148**, 1772 (1909).

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E. L. R. STOKSTAD

RECEIVED DECEMBER 26, 1951

THE BIOSYNTHESIS OF SQUALENE AND CHOLESTEROL[1]

Sir:

Squalene from shark liver oil was shown to be a dihydrotriterpene in 1926.[2,3] Although the structure of cholesterol was then only incompletely known, the suggestion was made that squalene might be an intermediate in steroid biosynthesis.[2,4] Balance studies which were carried out gave conflicting results.[5,6] Work carried out with isotopic tracers during recent years has demonstrated that acetate is the principal carbon source of cholesterol.[7,8] The distribution of acetate carbon which was found in the cholesterol molecule, led to the suggestion that cholesterol biosynthesis might proceed via the condensation of isoprenoid units.[9] The data were also compatible with a cyclization of squalene to cholesterol as proposed by Robinson.[10]

It has now been shown that squalene is synthesized biologically from acetate, that squalene is absorbed from the gut, and that carbon from labeled squalene is efficiently incorporated into cholesterol. Rat tissues do not contain detectable quantities of squalene. However, when the hydrocarbon is fed, a small amount can subsequently be recovered from the liver and intestinal tract. Rats received in their diet 0.5 g. of squalene and 0.54 millicurie of 1-C^{14} acetate (0.125 g.) per 100 g. rat per day for two days. The combined nonsaponifiable fractions of the livers and intestinal tracts were chromatographed on alumina and "washed out" with normal cholesterol. This yielded 35 mg. of hydrocarbon, having a specific activity of 2080 c.p.m. A portion was diluted with purified natural squalene[11] and two isomeric hexahydrochlorides,[2] m.p. 108° and 144° were prepared. Corrected for dilution, these derivatives had specific activities of 2120 c.p.m. and 2040 c.p.m., respectively. This demonstrated that all of the radioactivity of the hydrocarbon fraction resided in the squalene. The remainder of this C^{14} squalene was fed to mice at a level of 10 micromoles of squalene per animal per day for two days. Cholesterol and fatty acids were isolated from tissues. Data from one of two identical experiments are shown in Table I.

TABLE I

FEEDING OF C^{14} SQUALENE, 2080 C.P.M.,[a] TO MICE

1. Liver and gut	C^{14}, c.p.m.	% of squalene C recovered	RIC[b]
Cholesterol digitonide	132[c]	4.2	6.4
Cholesterol dibromide	131		
Fatty acids	<2		
2. Carcass and viscera			
Crude steroids	34		2.1
Cholesterol digitonide	43[c]	3.9	
Cholesterol dibromide	42		
Fatty acids	0		
		8.1	

[a] All C^{14} values expressed as c.p.m. of infinitely thick $BaCO_3$ samples. [b] RIC = (c.p.m. of cholesterol/c.p.m. of squalene fed) × 100. [c] Calculated for free cholesterol.

Comparison with earlier results indicates that the utilization of squalene carbon for cholesterol formation is 10–20 times as efficient as that of acetate.[7,8] It is also more than three times as efficient as that of isovaleric acid,[12] until now the most efficient carbon source of cholesterol. The percentage recovery of squalene carbon in cholesterol is based on the total amount fed. Since squalene is not quantitatively absorbed from the gut,[5] this figure (8%) represents a minimal value. The insignificant isotope concentration in the fatty acids precludes the possibility that squalene was

(1) Supported by a grant from the Life Insurance Medical Research Fund.

(2) I. M. Heilbron, E. D. Kamm and W. M. Owens, *J. Chem. Soc.*, 1630 (1926).

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(4) H. J. Channon, *Biochem. J.*, **20**, 400 (1926).

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(6) T. Kimizuka, *J. Biochem. (Japan)*, **27**, 469 (1938).

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(9) J. Würsch, R. L. Huang and K. Bloch, *ibid.*, in press.

(10) R. Robinson, *J. Soc. Chem. Ind.*, **53**, 1062 (1934).

(11) Generously supplied by Dr. Stanley Ames, Distillation Products Company.

(12) I. Zabin and K. Bloch, *J. Biol. Chem.*, **185**, 131 (1950).

converted to cholesterol by way of 2-carbon intermediates.

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RECEIVED FEBRUARY 9, 1952

(13) U. S. Public Health Service Research Fellow.

THE STRUCTURE OF THE ANTIBIOTIC MYCOMYCIN

Sir:

We have deduced the structure of the highly unstable antibiotic mycomycin[1] as 3,5,7,8-tridecatetraene-10,12-diynoic acid (I).

$$HC{\equiv}C{-}C{\equiv}CH{=}C{=}CHCH{=}CH{-}CH{=}CHCH_2CO_2H$$

Mycomycin undergoes an unusual rearrangement in normal aqueous potassium hydroxide at 27° involving an allene to acetylene isomerization accompanied by migration of existing acetylenic bonds. The rearranged acid, isomycomycin, has been assigned the structure 3,5-tridecadiene-7,9,11-triynoic acid (II).

$$CH_3{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2{-}CO_2H$$

I crystallizes in colorless needles from methylene chloride at −40°; m.p. 75° (dec. explosively) and $[\alpha]^{25}D$ −130° (*c*, 0.4, ethanol). (*Anal.* Calcd. for $C_{13}H_{10}O_2$: C, 78.76; H, 5.08; one C-methyl, 7.6; neut. equiv., 198; Found: C, 78.17; H, 5.36; C-methyl (Kuhn–Roth), 0.48; neut. equiv., 200). Ultraviolet absorption in diethyl ether: $\lambda^{m\mu}_{infl.}$ 256, ϵ 35,000; $\lambda^{m\mu}_{max.}$ 267, ϵ 61,000; $\lambda^{m\mu}_{max.}$ 281, ϵ 67,000.

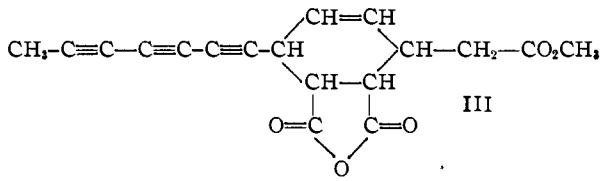
II crystallizes in colorless needles from ether–hexane, decomposes slowly above 140° and is optically inactive. (*Anal.* Found: C, 78.87; H, 5.43; C-methyl (Kuhn–Roth), 9.6; neut. equiv., 198). Ultraviolet absorption in diethyl ether: $\lambda^{m\mu}_{infl.}$ 246, ϵ 24,000; $\lambda^{m\mu}_{max.}$ 257.5, ϵ 58,000; $\lambda^{m\mu}_{max.}$ 267, ϵ 110,000; $\lambda^{m\mu}_{max.}$ 287.5, ϵ 14,000; $\lambda^{m\mu}_{max.}$ 305.5, ϵ 27,000; $\lambda^{m\mu}_{max.}$ 324, ϵ 41,000; $\lambda^{m\mu}_{max.}$ 347, ϵ 34,000.

Complete hydrogenation of I and II requires eight moles of hydrogen, quantitatively yielding *n*-tridecanoic acid, unequivocally identified by comparisons with an authentic sample.[2]

The infrared spectrum of I in dioxane has characteristic bands near 3180, 2200, 1930 and 1730 cm.$^{-1}$ attributed to ≡C—H, disubstituted —C≡C—, —CH=C=CH— and unconjugated —CO_2H[2a] functions, respectively. I reacts with acetylenic hydrogen reagents such as alcoholic silver nitrate. In view of the linear nature of the reduction product, the high order of optical activity of I can only be reconciled with its allenic function. The fine structure spacing ($\Delta\nu'$, 1900 cm.$^{-1}$) of the ultraviolet absorption maxima of I gives evidence that the two recognized acetylenic bonds are in conjugation.[3] The remaining two units of unsaturation are believed to be a conjugated diene, conjugated with the allenic group but not with the carboxyl group.

The location of the observed long wave length ultraviolet absorption maximum of I at 281 mμ limits the length of contributing conjugated multiple bonds to three units, part of which may be acetylenic.[4] The allene bond in the proposed 7,8-position of I performs a unique chromophoric role whereby the Δ^7 bond and the Δ^8 bond are conjugated with the 3,5-diene and 10,12-diyne, respectively, while the central carbon atom of the allene serves as an electronic insulator between the two resulting conjugated systems.[5] The two effectively isolated chromophores, each totaling three units of conjugation, explain the observed general light absorption zone of I.

The infrared spectrum of II in dioxane exhibits characteristic bands at 2200 cm.$^{-1}$ and 1730 cm.$^{-1}$ attributed to disubstituted —C≡C— and unconjugated —CO_2H,[2a] respectively. Monosubstituted acetylenic and allenic bands are absent. II analyzes for one C-methyl group, whereas I possesses none. II does not react with alcoholic silver nitrate, substantiating the absence of a ≡C—H function. The ultraviolet light absorption properties of II prove to be very similar to those recorded for compounds containing a conjugated dienetriyne grouping.[6,7] II reacts with ethereal diazomethane to form a methyl ester, crystallized from ether–hexane as colorless needles, m.p. 69–70°. A Diels–Alder reaction of the methyl ester of II with maleic anhydride gives a monoaddition product (III), crystallized from acetone–hexane as colorless plates, m.p. 177–178° (dec.). (*Anal.* Calcd. for $C_{18}H_{14}O_5$: C, 69.67; H, 4.55; CH_3O, 10.00. Found: C, 69.59; H, 4.64; CH_3O, 10.89.) Ultraviolet light absorption in methanol: $\lambda^{m\mu}_{infl.}$ 215, ϵ 82,000; $\lambda^{m\mu}_{max.}$ 272.5, ϵ 450; $\lambda^{m\mu}_{max.}$ 289, ϵ 430; $\lambda^{m\mu}_{max.}$ 310, ϵ 170. These light absorption properties of III



III

bear striking resemblance to the unique light absorption behavior of the conjugated triacetylene grouping when it is the sole contributing chromo-

(1) E. A. Johnson and K. L. Burdon, *J. Bact.*, **54**, 281 (1947).

(2) Obtained through the courtesy of Dr. H. J. Harwood of Armour and Company.

(2a) In a comparison of infrared spectra determined in dioxane solution, the location of the C=O stretching frequency exhibited by I and II near 1730 cm.$^{-1}$ is the same as the corresponding band of their respective perhydro derivatives and authentic *n*-tridecanoic acid. If I and/or II contained a conjugated carboxyl group, this band would be expected to occur at a measurably lower frequency than the corresponding saturated derivative.

(3) T. Brunn, C. J. Haug and N. A. Sorensen, *Acta Chem. Scand.*, **4**, 850 (1950).

(4) I. M. Heilbron, E. R. H. Jones and R. A. Raphael, *J. Chem. Soc.*, 268 (1943); I. M. Heilbron, E. R. H. Jones and F. Sondheimer, *ibid.*, 1586 (1947).

(5) In a somewhat analogous situation, the ultraviolet light absorption of tetraphenylallene ($\lambda^{m\mu}_{max.}$ 267, ϵ 12,000) corresponds to that of 1,1-diphenylethylene ($\lambda^{m\mu}_{max.}$ 250, ϵ 11,000) and not to *trans,trans*-diphenyl-1,3-butadiene ($\lambda^{m\mu}_{max.}$ 328, ϵ 56,000).

(6) K. Stavholt and N. A. Sorensen, *Acta Chem. Scand.*, **4**, 1567 (1950).

(7) E. R. H. Jones, M. C. Whiting, J. B. Armitage, C. L. Cook and N. Entwistle, *Nature*, **168**, 900 (1951).

phore.[8,9] More detailed accounts of the above structural considerations will appear in subsequent publications.

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(9) E. R. H. Jones, personal communication.

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RECEIVED FEBRUARY 28, 1952

HYPOTENSIVE ALKALOIDS OF VERATRUM ESCHSCHOLTZII

Sirs:

In view of the present interest in the Veratrum Alkaloids as hypotensive agents, we wish to report the isolation from *Veratrum eschscholtzii Gray* of neogermitrine,[1] and a new alkaloid, for which we propose the name escholerine.

Preliminary fractionation of a crude chloroform extract which was based on the selective solubilities of the alkaloids and their salts, in conjunction with assays[2] for hypotensive activity in anesthetized dogs, yielded an amorphous fraction that accounted for the major part of the hypotensive activity in the crude extract. Further fractionation by two 8-plate Craig countercurrent distributions[3] yielded two fractions, A and B, each with a high hypotensive activity. Fraction A was resolved on a 24-plate distribution using 2 *M* acetate buffer at *p*H 5.5 and benzene as the solvent system. Neogermitrine was obtained from the material recovered from tubes 8 to 13 by crystallizing from acetone–water (m.p. 234–234.8 (cor.)); $[\alpha]^{25}D$ −79 ± 2°, (*c* 0.9 in pyridine); the sample was further identified by comparison of its infrared spectrum, and by a mixed melting point with an authentic sample of neogermitrine from *Veratrum viride Aiton* kindly provided by Dr. J. Fried.

Fraction B was distributed on a 24-plate Craig apparatus, using 0.5 *M* acetate buffer *p*H 5.0 and benzene–cyclohexane 25:75 as the immiscible phases. The material recovered from tubes 8 to 13, when crystallized from acetone–water, yielded escholerine (m.p. 235–235.3 with dec. (cor.); $[\alpha]^{25}D$ −30 ± 2° (*c* 1.0 in py.); +7 ± 2° (*c* 1.0 in $CHCl_3$)). The analytical data indicate the empirical formula $C_{41}H_{61}O_{13}N$; (calcd. C, 63.46; H, 7.92; N, 1.80; eq. wt., 775.9; found: C, 63.42, 63.59; H, 8.00, 7.97; N, 2.04; eq. wt., 782, 772; picrate, m.p. 259.5° (dec.), $(C_{41}H_{61}O_{13}N \cdot HOC_6H_2(NO_2)_3$: C, 56.17; H, 6.42; found: C, 56.41; H, 6.38); aurichloride, m.p. 191.4° (frothing), $(C_{41}H_{61}O_{13}N \cdot HAuCl_4$. C, 44.13; H, 5.60; Au, 17.67; found: C, 44.53; H, 5.61; Au, 17.21). Volatile acid determination, found: 3.7 equivalents of acid.

Hydrolysis of escholerine with 0.1 *N* methanolic potassium hydroxide afforded acetic acid, α-methylbutyric acid and a base that has so far resisted all attempts at crystallization. A mixture of the *p*-phenylphenacyl esters of the acids after chromatography on a silicic acid[4] column, yielded *p*-phenylphenacyl acetate, m.p. 110.8–111.2° (calcd. C, 75.58; H, 5.55; found, C, 75.38; H, 5.66) and *p*-phenylphenacyl α-methylbutyrate, m.p. 70–71° cor. (calcd. C, 77.01; H, 6.80; Found: C, 76.61; H, 6.80).

The hypotensive activity[5] of neogermitrine and escholerine in anesthetized dogs was found to be 0.13 μg. [0.12–0.15] and 0.30 μg. [0.26–0.36], respectively.

The isolation procedure has, in addition, yielded the alkaloids isorubijervine, jervine, rubijervine, pseudojervine and veratramine, already known as constituents of *Veratrum viride*, and small amounts of four apparently new crystalline alkamines and a new ester alkaloid which will be described more fully in a subsequent publication.

(1) J. Fried and P. Numerof, Abst. 119th meeting A.C.S., Cleveland, Ohio, April, 1951, 12L.
(2) Assays were run according to the method of G. L. Maison and J. W. Stutzman, *Arch. Int. Pharmacodyn.*, **85**, 357 (1951). Evaluations were made at Boston University School of Medicine, Boston, Mass.
(3) J. Fried, H. White and O. Wintersteiner, THIS JOURNAL, **72**, 4621–4630 (1950).
(4) J. G. Kurchner, Arthur H. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **18**, 31 (1946).
(5) Expressed as micrograms per kilogram of dog per minute required for a ten minute intravenous infusion to lower the mean arterial blood pressure 30% when administered according to the method of G. L. Maison and J. W. Stutzman.[2] The bracketed numbers express the 95% confidence limits.

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W. MALESH

RECEIVED JUNE 18, 1951

MICROBIOLOGICAL OXYGENATION OF STEROIDS AT CARBON 11

Sir:

It is generally acknowledged that the most difficult series of steps in the partial synthesis of cortisone is that concerned with the introduction of oxygen at carbon 11 of the steroid nucleus.[1,2,3,4] We wish to report the oxygenation of steroids, *e.g.*, progesterone, at carbon 11 in a single step by means of common molds of the order *Mucorales* after a transformation period of 24–48 hours, in a lactalbumin digest–dextrose–cornsteep medium. Thus, from progesterone, a new 11-oxygenated steroid intermediate is made available for conversion to the cortical hormones. In these studies we have made use of the procedure of Zaffaroni, *et al.*,[5] for characterization of the transformation products.

The ability of several micro-organisms to oxidize a hydroxyl group or reduce a ketone group in a steroid is well recognized,[6] but heretofore the only microbiological oxygenation of a steroid carbon atom was reported by Krámli and Horváth[7] in the

(1) B. F. McKenzie, V. R. Mattox, L. L. Engel and E. C. Kendall, *J. Biol. Chem.*, **173**, 271 (1948).
(2) E. M. Chamberlain, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Aliminosa, R. L. Erickson, G. E. Sita and Max Tishler, THIS JOURNAL, **73**, 2396 (1951).
(3) L. F. Fieser, J. E. Herz and Wei-Yuan Huang, *ibid.*, **73**, 2397 (1951).
(4) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3546 (1951).
(5) A. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950).
(6) M. Welsch and C. Heusghem, *Compt. rend. soc. biol.*, **142**, 1074–1076 (1948).
(7) A. Krámli and J. Horváth, *Nature*, **160**, 639 (1947); **163**, 219 (1949).

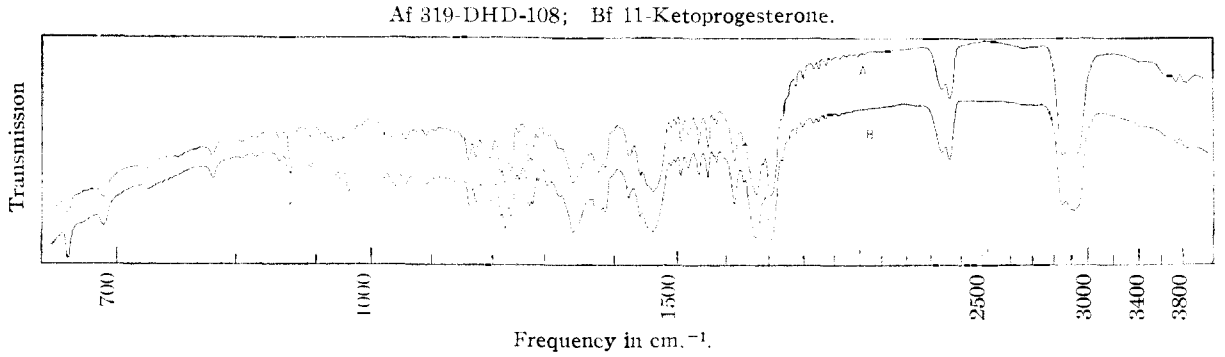
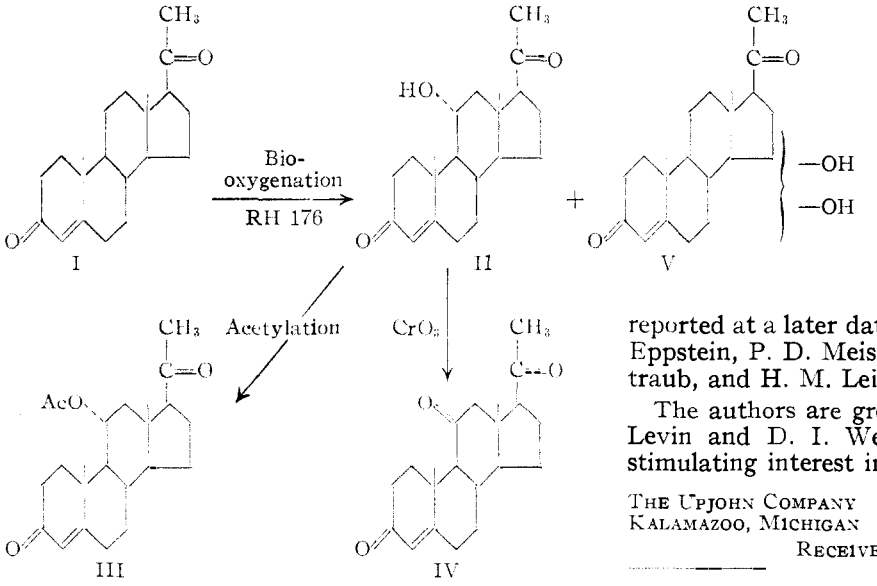


Fig. 1.—Infrared absorption spectra, liquid hydrocarbon mull, Perkin–Elmer Model 12C spectrophotometer: A, oxidation product of II; B, authentic 11-ketoprogesterone.

formation of a 7-hydroxycholesterol from cholesterol by *Proactinomyces roseus* or an *Azotobacter*.

After 1.0 g. of progesterone (I) had been subjected to the action of a species of *Rhizopus, e.g., Rhizopus arrhizus* (our strain RH 176), it was possible to isolate from the methylene chloride extract two new compounds. The first of these was a dihydroxyprogesterone (V), m.p. 245–248° (all melting points are uncorrected), $[\alpha]^{24}D$ +144° (pyridine)[8]; (*Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.74. Found: C, 72.83; H, 8.67) in 2.3% yield. Acetylation of V yielded a diacetate (VI), m.p. 145–148° and 153–154°; $[\alpha]^{23}D$ +71° (absolute ethanol); (*Anal.* Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.80; H, 7.82). Infrared studies confirmed that V was a dihydroxyprogesterone and VI the diacetate of V. Further characterization of this compound will be reported at a later date.



Alumina chromatography of the concentrate from the mother liquors from V gave an additional 5% of V and a fraction which yielded a monohydroxyprogesterone (II), m.p. 166–167°, $[\alpha]^{25}D$ +179° (chloroform); $\lambda_{max.}^{EtOH}$ 242 mμ, (log *E* 4.19); (*Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.40; H, 9.15. Found: C, 76.77; H, 8.92) in 10% yield. From II was formed a monoacetate (III), m.p. 175–177°, $[\alpha]^{24}D$ +143° (acetone); (*Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.33; H, 8.78).

Oxidation of the monohydroxyprogesterone (II) gave 11-ketoprogesterone (IV), m.p. 174–176°; $[\alpha]^{25}D$ +227° (chloroform); (*Anal.* Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.59; H, 8.55) identical in all respects with an authentic sample of 11-ketoprogesterone.[9]

Figure 1 shows a comparison of the infrared curves of the two 11-ketoprogesterones.[10]

Since Compound II has different physical properties than 11β-hydroxyprogesterone[11] and readily forms an acetate, it is concluded that Compound II is 11α-hydroxyprogesterone.

Similar microbiological oxygenations at carbon 11 using molds of the *Mucorales* order have been achieved on other steroid substrates, including androstenedione, 11 - desoxy - 17 - hydroxycorticosterone (Reichstein's substance S), and 11-desoxycorticosterone. Complete details of this work will be reported at a later date in collaboration with S. H. Eppstein, P. D. Meister, L. M. Reineke, A. Weintraub, and H. M. Leigh of our Laboratories.

The authors are greatly indebted to Drs. R. H. Levin and D. I. Weisblat for their helpful and stimulating interest in this work.

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KALAMAZOO, MICHIGAN
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H. C. MURRAY

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(8) We are grateful to W. A. Struck and his group for all rotations and microanalyses.

(9) Kindly supplied by Dr. T. F. Gallagher, of the Sloan–Kettering Institute for Cancer Research, New York, N. Y.

(10) We are indebted to Dr. and Mrs. J. L. Johnson, Mrs. G. S. Fonken and L. Scholten for ultraviolet, infrared and X-ray diffraction studies.

(11) T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, **23**, 684 (1940).